KINETIC MODEL OF HEXANOIC ACID PRODUCTION BY *CLOSTRIDIUM KLUYVERI* SHOWS PRODUCT TOXICITY EVEN AT NEUTRAL PH

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Hexanoic acid (Hex) - a six-carbon medium chain carboxylic acid - is a potential intermediate for bio-production of materials and fuels. Production of Hex through the reverse β-oxidation pathway by *Clostridium kluyveri* has been studied extensively, but much remains unknown about the kinetics of the process. A novel technique for high-throughput anaerobic growth experiments was developed, enabling the elucidation of substrate (ethanol and acetic acid) affinity and inhibition constants of *C. kluyveri* as well as product (butyric acid and Hex) inhibition constants. Using an expanded Monod-kinetic model, *C. kluvyeri* was found to have a μ_{max} of 0.281±0.019 h^{-1}, an affinity constant (K_S) for acetate of 5.3±1.4 mM and a threshold inhibition concentration (K_I) for acetate was put at 330 mM. Hexanoate, the main product, has a clear linear inhibition effect with a K_{Hex} of 0.011±0.001 mM Hex^{-1}, corresponding with a threshold concentration of 89.8±11.5 mM hexanoate, at neutral pH. No affinity effect was observed for ethanol, only a linear inhibition effect between 0 and 1 M ethanol, with a K of 0.404±0.143 M $ethanol^{-1}$. For butyrate, also no affinity effect was observed, but a K_I of 133.0±1.5 mM butyrate was determined. Yield was determined to be 0.060±0.017 g biomass.g $ethanol^{-1}$. This study provides fundamental insights in the kinetic behaviour of *C. kluyveri* as well as information about its potential use as a bio-catalyst for production of Hex, through a newly developed framework for high-throughput analysis of growth kinetics for anaerobic micro-organisms, specifically pure strains, but in the future potentially for mixed cultures as well.